The paper copy of the substitute sequence listing was printed from the floppy disk. The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy.

The substitute copy of the sequence listing is submitted to correct an inadvertent error in the Substitute Sequence Listing submitted on November 28, 2001. In SEQ ID NO:1 of the formal sequence listing, at nucleotide position 62, the correct nucleotide residue "A", which was originally disclosed in the application, was replaced by an incorrect residue "T" due to a typographical error. This produced the codon "ATT", translated as "Ile" at position 21 in the amino acid sequence of SEQ ID NO:2. The enclosed substitute copy of the Sequence Listing and diskette corrects this error in both SEQ ID NO:1 and SEQ ID NO:2 and changes the residue at position 62 to the correct "A" and the resulting codon into Asn. Support for this correction is found, for example, in the RapLR1 sequence on page 3 of the informal Sequence Listing labeled "SEQ ID NO:1/2", which is a part of the PCT application PCT/US99/06641 as filed.

With entry of the current amendment, claims 17-19, 28-30, 40, 43, and 44 were cancelled. Thus, claims 1-6, 20-27, 31-39, 41, and 42 are pending in the application. For convenience, a copy of the pending claims is attached hereto.

Claims 34-44 were examined in the Office Action mailed November 6, 2003.

The rejections will be addressed in the order presented in the Office Action mailed November 6, 2003.

Withdrawal from examination of claims 1-19

The Examiner alleges that claims 1-19 do not read on the elected species and has therefore withdrawn the claims from examination. Applicants respectfully traverse. SEQ ID NO:2 in the application as filed (*see, e.g.,* pages 1 and 3 of the listing of sequences in the application as filed in the PCT) clearly has the asparagine at position 21 as set forth in claim 1. Accordingly, SEQ ID NO:2 is encompassed by the claim. As

noted above, an inadvertent error was introduced into the electronic copy of the sequence listing provided to the patent office. The electronic copy has now been corrected. Applicants therefore respectfully request that claims 1-19 be examined.

Rejections under 35 U.S.C. §112, second paragraph

Claims 35, 36, and 44 were rejected as allegedly indefinite. The rejection as applied to claim 44 is moot in view of the cancellation of the claim. The rejection alleges that the recitation of the term "anti-neoplast" in claims 35 and 36 is unclear.

To the extent that the rejection applies to the amended claims, Applicants respectfully traverse. Webster's College Dictionary defines the adjective "antineoplastic" as "destroying, inhibiting, or preventing the growth of tumors. The word is also a noun meaning "an antineoplastic substance". Applicants maintain that the context of usage of the term "an antineoplast" makes clear that it refers to "an antineoplastic". However, in order to expedite prosecution, Applicants have amended "an antineoplast" to read as "an antineoplastic". Applicants therefore respectfully request withdrawal of the rejection.

Rejections under 35 U.S.C. § 112, first paragraph-Claims 34-37

Claims 34-37 were rejected as allegedly not enabled. In particular, the rejection alleges that the specification does not teach that the claimed pharmaceutical composition can be used *in vivo*.

Applicants respectfully traverse. The application clearly teaches how to make and used the claimed pharmaceutical compositions: the application teaches how to identify and/or design RNases of the invention (*see, e.g.,* page 14, lines 21-32; page 15, line 22 through page 16, line 2; page 16, lines 8-16 and lines 19-23; and page 18, lines 2-29) and how to produce and purify the ribonuclease (*see, e.g.,* the section starting at page 19, line 2 through page 29, line 26). The application teaches how to produce conjugate molecules, either chemically (*see, e.g.,* page 29, line 29 through page 31, line 31) or recombinantly (*see, e.g.,* page 32, line 2 through page 33, line 21); how to formulate pharmaceuticals (*see, e.g.,* page 35, line 18 through page 36, line 10); and how to

administer the pharmaceuticals (*see, e.g.*, page 36, lines 11-29). Accordingly, Applicants have taught how to make and use the invention.

The Examiner appears to be concerned that, despite Applicants' teachings in the specification, the cytotoxic reagents may not work *in vivo*. To provide further evidence of the *in vivo* utility of the cytotoxic reagents of the invention, Applicants submit herewith a Declaration under 37 C.F.R. § 132 by Susanna Rybak. A signed copy of the Declaration will be submitted in a Supplemental Amendment. Applicants apologize for any inconvenience to the Examiner.

In the Declaration, Dr. Rybak presents data using art-accepted animal models that demonstrate the anti-tumor effect *in vivo* of the RNases of the invention, either alone or when linked to a binding moiety. The experiments described in the Declaration use an exemplary RNase, rapLR1 (*see, e.g.*, page 14, lines 21-24), and immunoconjugates comprising rapLR1. The first set of experiments (paragraph 4) shows that thse cytotoxic reagents reduce lung metastasis. RapLR1 and an immunoconjugate comprising rapLR1 and the anti-MUC1 antibody (HMFG1) were prepared in accordance with the methodology described in paragraph 3. These reagents were tested for the ability to inhibit metastasis in a murine breast adenocarcinoma model. Animals that had been inoculated with breast adenocarcinoma cells received rapLR1 or HMFG1-rapLR1 conjugate. Control animals were injected with PBS or HMFG1 alone. The results (Exhibit B) showed that the number of metastases were reduced in those animals that received rapLR1 in comparison to controls. Moreover, the animals that received the immunoconjugates had even fewer metastases. Thus, these data demonstrate the anti-tumor effect of rapLR1 and HMFG1-rapLR1.

The effects of rapLR1 and HMFG1-rapLR1 were also tested on the growth of 4TI-MUC1 tumors that were implanted into the mammary fat pad of SCID mice (paragraph 5). Animals were administered rapLR1 or HMFG1-rapLR1 as described in paragraph 5. Control animals received PBS or HMFG1. The results (Exhibit C) show that rapLR1 by itself and when linked to HMFG1 reduces tumor growth.

Lastly, a rapLR1-anti-CD22 immunoconjugate (LL2-rapLR1) has also been tested *in vivo* in an aggressive model of disseminated murine lymphoma and shown to have anti-tumor activity. A copy of the paper describing these studies (Hursey *et al.*, *Leukemia and Lymphoma* 43:953-959, 2002) is attached to the Declaration as Exhibit D. Animals were inoculated with Daudi lymphoma cells and treated at various times, which are summarized in paragraph 6 of the Declaration, with PBS, LL2 plus rapLR1 or LL2-rapLR1 conjugate. The results presented in Table II, page 957 of the paper showed that those animals that received the immunoconjugate exhibited enhanced survival.

Thus, the Declaration presents *in vivo* evidence that the RNases of the invention and immunoconjugates comprising the RNases are effective anti-tumor agents in multiple models.

In view of the foregoing, Applicants respectfully request withdrawal of the rejection.

Rejections under 35 U.S.C. § 112, first paragraph-claim 38

Claim 38 was rejected as allegedly not enabled. The rejection alleges that the specification does not teach any way of targeting the cells other than as a conjugate to a ligand that binds a cell surface receptor. Applicants respectfully traverse. The Examiner appears to believe that the RNases of the invention cannot enter cells in the absence of a targeting moiety or some other method of treating the cells. However, as shown in the Declaration, no special treatment is required for the RNases of the invention to enter cells. Indeed, it is known in the art that RNase A family members can be internalized by cells (*see, e.g.*, page 993, first column, second to the last sentence of the first full paragraph immediately of Boix, *et al.*, *J. Mol. Biol.* 257:992-1007, 1996, which is reference "AG" in Applicants' IDS submitted October 2, 2001).

Rejections under 35 U.S.C. § 112, first paragraph-claim 44

The rejection is obviated by the cancellation of claim 44.

Rejections under 35 U.S.C. § 112, first paragraph-claims 39-43

Claims 39-43 were rejected as allegedly not enabled for a method of killing cancer cells using a cytotoxic reagent other than transferrin-receptor antibody linked to SEQ ID NO:2. The rejection with respect to claims 40 and 43 is obviated by the cancellation of those claims. With regard to the rejection of the other claims, Applicants respectfully traverse.

The Examiner contends that the specification only enables a cytotoxic reagent comprising transferrin receptor-linked to SEQ ID NO:2. As explained above, Applicants teach how to make the conjugates of the invention and how to administer them. Further, Applicants describe a variety of ligand binding moieties that may be used in preparing the conjugates (*see, e.g.*, page 13, lines 13-19 and page 35, lines 1-15). In addition, the field of immunoconjugates and targeting various moieties to particular cells is advanced, as evidence by the references listed in Applicants' IDS submitted October 2, 2001. Accordingly, one of skill would be able to select appropriate antibodies with a reasonable expectation that they would successfully target the RNase. Thus, the teachings in the specification in combination with that which is known in the art, guide the artisan in practicing the invention.

Lastly, the Declaration under 37 C.F.R. § 1.132 of Susanna Rybak presents additional evidence that the specification is enabling. The Declaration shows data demonstrating the anti-tumor activity *in vivo* of immunoconjugates comprising two different antibodies. Thus, multiple immunoconjugates comprising different antibodies have been shown to work.

In view of the foregoing, the claims are enabled. Applicants therefore respectfully request withdrawal of the rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

AMENDMENT TO THE SPECIFICATION:

Amendment at the paragraph starting at page 8, line 29 through page 9, line 29:

One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, J. Mol. Evol. 35:351-360 (1987). The method used is similar to the method described by Higgins & Sharp, CABIOS 5:151-153 (1989). The program can align up to 300 sequences of a maximum length of 5,000. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster can then be aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences can be aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program can also be used to plot a dendrogram or tree representation of clustering relationships. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison. Another example of algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in Altschul, et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information ~~(http://www.ncbi.nlm.nih.gov/)~~. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul, et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended

in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

AMENDMENTS TO THE CLAIMS:

35. (amended) The pharmaceutical composition of claim 34, further comprising an antineoplastic.

36. (amended) The pharmaceutical composition of claim 35, where said antineoplastic is Adriamycin.

PENDING CLAIMS

1. (as filed) A recombinant ribonuclease that has (a) measurable ribonuclease activity; (b) an amino terminal end beginning with a glutamine; (c) a leucine at position 11; an asparagine at position 21, a threonine at position 85, and a histidine at position 103, such positions being determined through alignment with reference to those specified amino acid positions of SEQ ID NO:2; and (d) substantial identity to SEQ ID NO:2.

2. (as filed) The recombinant ribonuclease of claim 1, further comprising a methionine residue at position 1.

3. (as filed) The recombinant ribonuclease of claim 2, wherein the methionine residue at position 23 as shown in SEQ ID NO:2 is replaced with a leucine residue.

4. (as filed) The recombinant ribonuclease of claim 3, further comprising histidine residues at 1 through 6 (SEQ ID NO:9).

5. (as filed) The recombinant ribonuclease of 1, wherein the glutamine at position 1 is cyclized to pyroglutamic acid.

6. (as filed) The recombinant ribonuclease of claim 1, wherein the glutamine at position 1 is replaced with a serine.

7. (as filed) A cytotoxic reagent comprising the recombinant ribonuclease of claim 1, linked to a ligand binding moiety.

8. (as filed) The cytotoxic reagent of claim 7, further comprising a methionine residue at position 1.

9. (as filed) The cytotoxic reagent of claim 8, wherein the methionine residue at position 23 as shown in SEQ ID NO:2 is replaced with a leucine residue.

10. (as filed) The cytotoxic reagent of claim 9, further comprising histidine residues at 1 through 6.

11. (as filed) The cytotoxic reagent of claim 7, wherein the glutamine at position 1 is cyclized to pyroglutamic acid.

12. (as filed) The cytotoxic reagent of claim 7, wherein the glutamine at position 1 is replaced with a serine.

13. (as filed) The cytotoxic reagent of claim 7, wherein the ribonuclease of SEQ ID NO:2 is linked to a ligand binding moiety through a covalent bond.

14. (as filed) The cytotoxic reagent of claim 13, wherein said covalent bond is at the carboxy terminus of the ribonuclease of SEQ ID NO:2.

15. (as filed) The cytotoxic reagent of claim 7, wherein said ligand binding moiety is an antibody directed against a cell surface antigen present on a cancer cell.

16. (as filed) The cytotoxic reagent of claim 15, wherein said antibody is a recombinant single chain antibody.

20. (as filed) A nucleic acid which encodes a recombinant ribonuclease having a nucleotide sequence as shown in SEQ ID NO:14 and conservative variants thereof.

21. (as filed) A recombinant ribonuclease encoded by a nucleic acid comprising SEQ ID NO:14 and conservative variants thereof.

22. (as filed) The ribonuclease of claim 21, wherein the amino acid sequence is selected from the group consisting of SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26.

23. (as filed) A cytotoxic reagent comprising the ribonuclease of claim 22 linked to a ligand binding moiety.

24. (as filed) The cytotoxic reagent of claim 23, wherein the ribonuclease is linked to a ligand binding moiety through a covalent bond.

25. (as filed) The cytotoxic reagent of claim 24, wherein said covalent bond is at the carboxy terminus of the ribonuclease.

26. (as filed) The cytotoxic reagent of claim 23, wherein said ligand binding moiety is an antibody directed against a cell surface antigen present on a cancer cell.

27. (as filed) The cytotoxic reagent of claim 26, wherein said antibody is a recombinant single chain antibody.

31. (as filed) A method of preparing a substantially pure recombinant ribonuclease, said method comprising:

(i) contacting said ribonuclease with an effective concentration of a cleaving agent such that the ribonuclease is cleaved after the carboxy group of methionine at position 1;

(ii) passing said ribonuclease through a Ni^{2+}-NTA agarose column; and

(iii) eluting said substantially pure ribonuclease from said column.

32. (as filed) A method of preparing a substantially pure recombinant cytotoxic reagent, said method comprising:

(i) contacting said cytotoxic reagent with an effective concentration of a cleaving agent such that the cytotoxic reagent is cleaved after the carboxy group of methionine at position 1;

(ii) passing the cytotoxic reagent through a Ni^{2+}-NTA agarose column; and

(iii) eluting said substantially pure cytotoxic reagent from said column.

33. (as filed)The method of claim 31, 32, wherein said cleaving agent is CNBr.

34. (as filed) A pharmaceutical composition comprising a ribonuclease expressed from recombinant DNA, said ribonuclease comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:24, and SEQ ID NO:26 in a pharmaceutically acceptable carrier.

35. (amended) The pharmaceutical composition of claim 34, further comprising an antineoplastic.

36. (amended) The pharmaceutical composition of claim 35, where said antineoplastic is Adriamycin.

37. (as filed) A pharmaceutical composition comprising a cytotoxic reagent, said cytotoxic reagent comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:24 and SEQ ID NO:26 in a pharmaceutically acceptable carrier.

38. (as filed) A method of killing cancer cells comprising contacting cells to be killed with a ribonuclease expressed by recombinant DNA and having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:24 and SEQ ID NO:26.

39. (as filed) A method of killing cancer cells comprising contacting cells to be killed with a cytotoxic reagent expressed by recombinant DNA, comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:24 and SEQ ID NO:26 covalently linked to a ligand binding moiety, said ligand binding moiety directed against a cell surface antigen on the cancer cells.

41. (as filed) The method of claim 39, wherein said ligand binding moiety is an antibody.

42. (as filed) The method of claim 41, wherein said antibody is a single chain antibody.